Synopsis

Influence of FtsH Protease on the Medial FtsZ ring in *Escherichia coli*

FtsH is an essential AAA family Zn^{++} metalloprotease of *Escherichia coli*, possessing ATPase-dependent chaperon activity and ATP-dependent protease activity. Heat shock transcription factor Sigma32, LpxC, SecY, and bacteriophage λ protein CII are some of the substrates of FtsH. Although FtsH is known to influence several cellular processes, the role of FtsH in bacterial cell division had not been identified. FtsZ is the principal cell division protein that marks the cell division site at mid-cell by forming a ring structure. Using a pair of *ftsH*-null and isogenic wild type strain of *E. coli*, earlier studies in the laboratory had demonstrated that proteolytic function of FtsH is required for the presence of FtsZ rings at mid-cell site. It was also shown that FtsZ is not a substrate for FtsH protease *in vivo*. In view of these observations, using a pair of *ftsH*-null and isogenic wild type strain of *E. coli*, experiments were carried out to find out the mechanism behind the requirement for FtsH protease for the presence of FtsZ ring at mid-cell site. Viability of the cells having *ftsH*-null status was maintained by a suppressor mutation at another locus, and was found to be comparable to that of isogenic wild type cells.

Immunostaining for FtsZ showed that only 20% cells of *ftsH*-null strain of *E. coli* has FtsZ ring at mid-cell site, On the contrary, more than 90% cells of isogenic wild type cells had FtsZ ring at mid-cell site. Live cell imaging with FtsZ-GFP also showed similar results. Low fraction of *ftsH*-null cells having FtsZ ring was found to be independent of slow growth rate of the cells. Confocal microscopy revealed that *ftsH*-null cells lacked the normal helical spiral-type structure of FtsZ, unlike the intact FtsZ helices present in isogenic wild type cells. FtsZ protein levels in the membrane and cytoplasmic fractions of *ftsH*-null cells were found to be same as those in the isogenic wild type cells. Exogenous expression of wild type FtsH in *ftsH*-null cells could restore FtsZ ring status to normalcy, similar to that in the isogenic wild type cells. However, this restoration could not be accomplished by FtsH mutants, which were lacking in ATP binding, ATPase, or protease activities.

FtsA anchors FtsZ to the membrane and a specific FtsZ/FtsA ratio is known to be critical for cell division. Further, FtsA and/or ZipA are required for the stabilisation of FtsZ ring at mid-cell site. The levels of FtsA were found to be lower by more than 2.5-fold in all the membrane and soluble fractions of *ftsH*-null cells. The levels of FtsA were found restored to normalcy upon complementation with exogenous expression of FtsH. Low levels of FtsA were not due to the slow growth of *ftsH*-null cells. Exogenous expression of FtsA or FtsA-GFP restored FtsZ in more than 90% of *ftsH*-null cells. Moreover, FtsA mutants, which are defective in the interaction with FtsZ, did not restore FtsZ rings to normalcy. The levels of ZipA were found to be same in *ftsH*-null and isogenic wild type cells. Expression of ZipA or ZipA-GFP could restore FtsZ rings to normalcy in *ftsH*-null cells. These data showed that low FtsA levels might be the reason for low percentage of cells having FtsZ ring in *ftsH*-null cells. It implied that *ftsH*-null cells might have been managing FtsZ ring stabilisation with ZipA, to facilitate septation.

Real time RT-PCR showed that the levels of *ftsA* mRNA and those of all the other *fts* genes, except *ftsZ*, in the 16-gene *dcw* cluster, were found to be low in *ftsH*-null cells. Moreover, real time RT-PCR using specific primers designed for multiple promoters of *ftsZ* and for the RNaseE processing site, just upstream of *ftsZ*, showed that the levels of transcripts of the genes upstream to RNaseE site were significantly low and that the levels of *ftsZ* transcripts, which were downstream to RNaseE site, were unaffected. On the contrary, the levels of mRNAs of *fts* genes, such as *ftsE*, *ftsX*, *ftsN*, and *zipA* that were located at another part of the genome, were normal in *ftsH*-null cells. These observations suggested that the reason for the low levels of FtsA protein might be low levels of *ftsA* mRNA. In addition, the low levels of other *fts* mRNAs from the *dcw* cluster, and probably of the respective proteins, might contribute to the slow growth of *ftsH*-null cells.

The *ftsH* null strains also showed less compact nucleoids and the nucleoids did not look bilobular. This data suggested that there may be some defect in the compaction of nucleoids in *ftsH*-null cells. On the contrary, isogenic wild type cells, when grown slow like the growth of *ftsH*-null cells, had no defect in nucleoid compaction and looked bilobular. The proper compaction of nucleoids could be restored only by wild type FtsH, but not by the protease mutant of FtsH. These observations suggest that proteolytic

activity of FtsH might be required for the proper compaction of nucleoids, which in turn might have influence on the placement of FtsZ ring at mid-cell site.

In parallel, different percentage of silver stained single-dimension SDS-PAGE showed conspicuous difference in the protein profiles of the membrane and soluble fractions of *ftsH*-null cells, in comparison to those of isogenic wild type cells. FtsZ co-immuno precipitation (CoIP) of total cell lysates of *ftsH*-null and isogenic wild type cells showed differential interaction of two proteins, the outer membrane protein A (OmpA) and a 50 kDa protein, between the two strains. The level of OmpA was 2.5-fold high in *ftsH*-null cells, in comparison to that in isogenic wild type cells. However, overexpression of *ompA* in isogenic wild type cells did not have any effect on FtsZ rings in isogenic wild type cells. Two-dimensional gel electrophoresis for membrane and soluble fractions of *ftsH*-null cells, in comparison with that of isogenic wild type cells, showed that several proteins in each fraction were either present or absent between these two strains. Most of these proteins were then identified using MALDI-TOF / LC – MS methods. Identification of these proteins, which were present differentially between *ftsH*-null and isogenic wild type cells, has revealed existence of many more hitherto unidentified potential substrates of FtsH and therefore cell processes, which FtsH may influence.